

-68-

CLAIMS

1. A method of modifying a producer cell which producer cell comprises integrated into its genome a provirus which provirus comprises one or more recombinase 5 recognition sequences within or upstream of its 3' LTR, the method comprising: introducing into the cell a construct comprising a 5' recombinase recognition sequence, an LTR and a 3' recombinase recognition sequence in that order, in the presence of a recombinase which is capable of acting on the recombinase recognition site(s) such that the nucleotide sequence between the 5' and 3' recombinase recognition sequences in the 10 construct is introduced into the provirus.
2. A method according to claim 1 wherein the construct further comprises at least one nucleotide sequence of interest (NOI) between the 5' recombinase recognition sequence and the LTR, which NOI is operably linked to a transcriptional regulatory 15 sequence.
3. A method according to claim 1 or claim 2 wherein the construct further comprises a 5'LTR and/or a packaging signal.
- 20 4. A method according to any one of claims 1 to 3 wherein the LTR is a heterologous regulatable LTR.
5. A method according to claim 4 wherein the regulatable LTR comprises an ischaemic like response element (ILRE).
- 25 6. A method according to any one of claims 1 to 3 wherein the LTR is inactive.
7. A method according to any one of the preceding claims wherein the provirus comprises an NOI encoding a selectable marker, which NOI is flanked by recombinase 30 recognition sites
8. A method according to any one of the preceding claims wherein the provirus comprises an internal 5' LTR upstream of the recombinase site or the 5' recombinase site

-69-

where there is more than one site.

9. A method according to any one of the preceding claims wherein the U3 region of the 5' LTR is inactive.

5

10. A method according to any one of the preceding claims wherein the U3 region of the 5' LTR and/or the U3 region of the second internal 5'LTR comprises a heterologous promoter.

10 11. A method according to any one of the preceding claims wherein the provirus comprises two recombinase recognition sites and as a preliminary step, the recombinase is expressed in a host cell such that the nucleotide sequence present between the two sites is excised.

15 12. A method according to any one of the preceding claims wherein the producer cell is a high titre producer cell.

13. A method according to any one of the preceding claims wherein the provirus is a lentivirus.

20

14. A method according to claim 13 wherein the lentivirus is HIV or EIAV.

15. A method according to any one of claims 2-14 wherein the provirus further comprises a second NOI.

25

16. A producer cell obtainable by the method of any one of claims 1 to 15.

17. An infectious retroviral particle obtainable from the producer cell of claim 16.

30

18. A derived producer cell comprising integrated into its genome a retroviral vector comprising in the 5' to 3' direction a first 5' LTR; a second NOI operably linked to a second regulatable 3' LTR; and a third 3'LTR;

-70-

wherein the third 3'LTR is positioned downstream of the second regulatable 3'LTR in the producer cell.

19. A producer cell according to claim 18 wherein the first 5' LTR comprising 5'R and 5' U5 sequences is derivable from a first vector; the second NOI operably linked to a second regulatable 3' LTR is derivable from a second vector; and the third 3'LTR is derivable from the first vector.
20. A producer cell according to claim 18 or claim 19 wherein the first vector comprises a retroviral vector wherein the retroviral vector comprises a first NOI flanked by recombinase recognition sequences.
21. A producer cell according to claim 19 or claim 20 wherein the retroviral vector further comprises an internal LTR located upstream of the first NOI and downstream of a packaging signal wherein the internal LTR comprises a heterologous U3 sequence linked to heterologous R and U5 sequences.
22. A producer cell according to any one of claims 18 to 21 wherein the third 3'LTR is transcriptionally quiescent.
23. A producer cell according to claim 22 wherein the third 3' LTR comprises a deletion in the U3 sequence.
24. A producer cell according to any one of claims 18 to 23 wherein the first NOI is a selectable marker.
25. A producer cell according to claim 19 wherein the second vector comprises a second NOI operably linked to a second regulatable 3'LTR comprising at least one recombinase recognition sequence.
26. A producer cell according to 25 wherein the second regulatable 3'LTR comprises a deletion in the U3 sequences in the 3'LTR.

-71-

27. A producer cell according to claim 25 or claim 26 wherein the second NOI comprises a coding sequence operably linked to a promoter.

28. A producer cell according to claim 27 wherein the second NOI comprises a  
5 discistronic construct.

29. A producer cell according to claim 28 wherein the discistronic construct comprises a therapeutic gene, an internal ribosomal entry site (IRES) and a reporter gene.

10 30. A method for producing a high titre regulatable retroviral vector, the method comprising the steps of:

(i) providing a derived producer cell comprising integrated into its genome a first vector;

15 (ii) introducing a second vector into the derived producer cell using a recombinase assisted method;

wherein the derived producer cell comprises a retroviral vector comprising in the 5' to 3' direction a first 5' LTR; a second NOI operably linked to a second regulatable 3' LTR; 20 and a third 3'LTR; wherein the third 3'LTR is positioned downstream of the second regulatable 3'LTR in the derived producer cell.

31. A method according to claim 30 wherein the third 3' LTR is transcriptionally active but expression is directed away from the second regulatable 3'LTR.

25

32. A method for introducing a second regulatable 3'LTR into a derived producer cell wherein the method comprises a recombinase assisted method.

33. A method according to claim 32 wherein the recombinase assisted method is a  
30 Cre/lox recombinase method.

-72-

34. A process for preparing a regulated retroviral vector as defined in claim 17 comprising performing the method according to any one of claims 30 to 33 and preparing a quantity of the regulated retroviral vector.

5 35. A regulated retroviral vector produced by the process according to claim 34.

36. A regulated retroviral vector according to claim 35 wherein the retroviral vector is capable of transducing a target site.

10 37. A regulated retroviral vector according to claim 36 wherein the retroviral vector is produced in sufficient amounts to effectively transduce a target site.

38. A regulated retroviral vector according to claim 36 or claim 37 wherein the target site is a cell.

15 39. A cell transduced with a regulated retroviral vector according to claim 38.

40. Use of a regulated retroviral vector according to any one of claims 35 to 38 in the manufacture of a pharmaceutical composition to deliver an NOI to a target site.

20 41. Use of a regulated retroviral vector according to any one of claims 35 to 38 in the manufacture of a medicament for diagnostic and/or therapeutic and/or medical applications.

25 42. Use of a recombinase assisted mechanism to introduce a regulated 3'LTR into a derived producer cell line to produce a high titre regulated retroviral vector.

43. A derived stable producer cell capable of expressing regulated retroviral vectors according to claims 35 to 38.

30 44. A derived stable producer cell according to claim 43 wherein the regulated retroviral vector is a high titre regulated retroviral vector.

Rule 12b

-73-

45 49. A nucleic acid molecule according to any one of claims 46 to 48 wherein the LTR is a heterologous regulatable LTR.

46 50. A nucleic acid molecule according to any one of claims 46 to 48 wherein the LTR is transcriptionally quiescent.

47 51. A method and/or a producer cell substantially as described herein and with reference to the accompanying Figures.

10

15

20

25

30

35

40

45

50